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TELEGRAM: "FOODSEARCH" MYSORE.

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CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE

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By Air-Mail

Dear Sir,

A few days ago, Dr.V.Subrahmanyan received a letter from Dr.D.L. Shrivastava intimating that you were anxious to know how the immunological experiments with elephants ~~you~~ you initiated here in January, 1952, were progressing. I have been asked to write to you in this matter.

In April last year, I had reported to you the results of these experiments which Dr.T.R. Doraiswamy and I jointly carried out. In the covering letter, I had also indicated that it would not be possible to do any further work on account of the unhelpful attitude taken by the authorities of the Mysore Forest Department. I wonder whether you received my letter or it gave the impression that we were still continuing with the experiments. Anyway, I am enclosing herein a copy of the report I had already sent you.

In spite of my personally meeting the Chief Conservator of Forests and explaining the object of our studies, it was not possible to get his concurrence to further experiments on elephants. He appears to have acted on the advice of the Veterinary Officers who tend to be over~~ly~~ cautious, particularly because these animals, as beasts of burden or otherwise, are valuable assets to the Government. Also they are none too certain that bleeding and injections of foreign protein are innocuous to the animals.

Moreover, the mahouts or keepers are, like Captains of ships, the real masters of the elephants. Each animal is assigned as the life-long ward to a mahout and becomes very much attached to him in course of time. And the mahouts are held responsible for the health and physical fitness of the animals in their charge. Being mostly uneducated, their minds are ridden with prejudices and superstitions. They believe that bleeding and injections will cause harm to the animals in the long run and undermine their strength. The Officers do not go very much against the wishes of these men. For, they will blame the experimental treatment should, at a later date, anything go wrong with the animals ^{care} ~~work~~ in the natural course of things. Even on the first day when you were here, you must have sensed the unwillingness of the mahout~~s~~ to allow us to take a small blood specimen from his animal. On later occasions, we had considerable trouble in coaxing them to agree to our taking blood samples from their wards. Thus ignorance and prejudice combined to stall the experiments so enthusiastically started. It was with the deepest regret and only because

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it could not be helped in the least, I finally decided to discontinue the experiments.

As for the results already obtained, those relating to antibody formation are inconclusive and have to be repeated whenever the opportunity arises in the future. Studies on complement have indicated that for some reason elephant blood serum shows low complement activity. Whether this is a true characteristic of the species or due to the complement or its essential components being bound to some blood constituent is to be determined. The latter may be a special feature of the elephant. I would like to know how this could be verified experimentally. I may mention here that in four animals whose blood picture we examined high leucocytic counts (14-24,000) were found, the count increasing with the age of the animal. I wonder whether these findings have a direct bearing on the low complement activity observed. Further experiments are needed to throw light on these points. I would earnestly solicit your valuable suggestions on these aspects. If you could outline any simple elucidative or confirmatory experiments, I will try to put them through.

Could the findings with regard to the complement be recorded in some suitable journal, if necessary, after some further work? They may serve as a basis, however preliminary, for any future experiments. At any rate, it will help to avoid needless repetition of the same type of experiments. I am hopeful that the co-operation and assistance of the Forest Department could be again secured for any further studies on the complement, provided large or repeated bleedings are not called for. We will take up the matter at this end after hearing from you.

I believe I have conveyed all the information you probably wanted. Should there be any points on which you require further clarification, I will gladly furnish the details.

Hoping to have your considered advice on the above, and with greetings and respectful regards,

Yours sincerely,

M.V. Lakshminarayan Rao
(M.V. Lakshminarayan Rao)

Encl/

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IMMUNOLOGICAL EXPERIMENTS WITH ELEPHANTS

(Carried out in Mysore - January-March, 1952)

(Initiated by Prof. M. Heidelberger)

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The object of the experiments was to study:

- (a) the complement activity of elephant blood, and
- (b) the formation of antibody in elephants to injected human γ -globulin.

The animals were made available through the courtesy and co-operation of the Forest Department, Government of Mysore.

Complement Activity:

Blood was drawn from the ear vein by means of a sterile canula and rubber tubing and collected in a sterile tube. It was preserved in an ice-bath and transported to the laboratory where the serum was separated by centrifugation. The serum was stored at deep-freeze until required for test. All tests were carried out within 24 hours after drawing the blood and in some cases immediately after the serum was separated. For the quantitative estimation of complement activity the method described in Kabat & Meyer's "Experimental Immuno Chemistry (1948)" was closely followed.

The results are tabulated below:

Name of elephant.	Sex	Approximate Age	Complement Activity (100 per cent lytic units)
BGR	♂	65 years	40
HYG	♂	60 "	30
HNS	♂	55 "	25
TRN	♀	50 "	40
PAD	♀	45 "	35
LID	♀	30 "	20
LEN	♀	25 "	40
LTH	♀	25 "	35
SAR	♂	12 "	30

(♂ = Bull, ♀ = Cow)

Guinea pig serum = $\frac{440}{420}$

The blood complement activity of the elephants tested ~~(prepared in the laboratory)~~ appeared to be very low as compared to that of the guinea pig.

In the case of three elephants (~~BGR~~^{BGR}, HNS and TRN), the tests were repeated, the blood being immediately transported in ice to the laboratory (within 20 minutes after bleeding) for serum separation and testing. The activity, however, was found to be the same as before.

Normal saline extracts of the clot after serum separation were tested and found to have little residual activity.

The serum of elephants when mixed with that of the laboratory guinea pig serum did not affect the activity of the latter.

The experiments made it ~~appear~~^{clear} that the observed low complement activity of elephant blood serum was not due to loss during transport and the short periods of cold storage or the holding up of complement by the blood clot, nor to the presence of any anti-lytic factors. Based on these results, the obvious inference seems to be that the complement content of elephant blood is itself low. A possible alternative is that the complement or some of its essential components are firmly bound to some blood constituent which may be a special feature of elephant blood, at any rate, of the animals examined.

Antibody Formation:

Two bull elephants, BGR (♂ 65 years) and HNS (♂ 60 years) were injected 20 cc. intravenously and 25 cc. subcutaneously of a sterile 20 per cent solution of human γ -globulin in physiological saline. Blood specimens were drawn from both animals prior to the injection.

The second one (HNS) went into 'heat' a few days after the injection and became unmanageable so far as bleeding was concerned. Observations were therefore confined to the other animal only.

As the Veterinary Officers as well as the keepers in charge of the elephants had strong objection to any large bleedings only small specimens were drawn for test purposes at weekly intervals. The observations were continued for six weeks, the fifth bleeding being taken a fortnight after the fourth.

Qualitative tests indicated that antibody formation
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~~Commenced~~
~~communicated~~ after the first week, reached a maximum at the end of the third week and tended to fall off thereafter. At the end of the six weeks, the test was weakly positive. Quantitative estimation (according to procedures given in Kabat and Meyer's book) showed that the serum antibody content at maximum response corresponded to 0.15 mg N/ml.

By the time we completed 4 weeks' observation, the local Forest Officer informed us that we would not be permitted to carry out any further experimental bleeding or treatment. The last bleeding was a matter of special concession to us. Therefore the desensitization studies could not be put through. We approached the Chief Conservator of Forests with the request that he may please place at our disposal at least one or two animals for continuing the studies. We received the categorical reply that as the Veterinary Officers were not certain that the proposed experimental treatment and repeated bleedings would be innocuous to the ultimate health and physical fitness of the animals, the request could not be acceded to. The experiments had, therefore, to be regretfully discontinued with the above, rather preliminary, observations.
